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PAPER

Uptake and bioavailability of persistent organic pollutants by plants grown in contaminated soil

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This paper assesses the uptake of persistent organic pollutants (POP's) into plants. In particular, uptake of α-endosulfan, β-endosulfan and endosulfan sulfate from lettuce. The lettuce plants were grown on compost that had previously been contaminated at 10 and 50 μg g^{-1} per POP. The soil was slurry spiked by adding the appropriate amount of POP in acetone in an approximate ratio of 1 : 2, w/v soil : solvent. The solvent was left to evaporate at ambient temperature for 24 hours. Lettuce plants were grown under artificial daylight for 12 hours a day. The influence of soil ageing on the recovery of POP's from spiked soil samples was also assessed. The average recovery of endosulfan compounds from slurry spiked soil (10, 20 and 40 μg g^{-1}) was consistent (92.9 ± 4.4% for $n = 9$). However, ageing of endosulfan compounds on the slurry spiked soil resulted in lower recoveries (average losses were 12.5% after 14 days ageing of slurry spiked soil). The uptake of POP's was assessed by measuring the amount of endosulfan compounds in roots and leaves from lettuce plants after 10, 20 and 33 days. In addition, control plants grown in uncontaminated soil were monitored and analysed. It was found that endosulfan compounds were present in the roots of all lettuce plants irrespective of soil spike level or age of plant. In the 33 day lettuce plants where the soil was spiked at the highest level (50 μg g^{-1}) endosulfan compounds were determined in the leaves. The root to leaf ratio was found to be 3.1 for α-endosulfan, 46.0 for β-endosulfan, and 24.3 for endosulfan sulfate. Spiked lettuce samples were subjected to *in vitro* gastrointestinal extraction to assess the bioavailability of endosulfan compounds. No detectable endosulfan compounds were determined in the gastric extracts while small quantities (range 0.06–0.12 μg g^{-1}) were found in the intestinal extraction. All samples (soil and lettuce) were extracted using pressurised fluid extraction and analysed using gas chromatography with mass selective detection.

1. Introduction

Huge amounts of organochlorine insecticides are used throughout the world for the control of a wide variety of pests in food and non-food crops.[1] These compounds are well-known as environmentally persistent organic pollutants (POP's) and tend to accumulate in wildlife due to their lipophilicity.[2] The majority of these insecticides *e.g.* DDT, are banned in terms of their useage in the developed world.[3]

Currently, endosulfan is one of the most common organochlorine insecticides used; it has been used as a DDT substitute, owing to its lower bioaccumulation and is widely employed in large amounts in many parts of the world.[4] Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide) comprises two stereoisomers, the α-isomer and β-isomer. This insecticide is used for a broad spectrum of pests as well as on several food crops such as tea, coffee, grains, fruits and vegetables; it has also been used as a wood preservative.[5,6] In spite of this, it is considered a highly toxic pesticide (toxicity class 1) according to the US Environmental Protection Agency.[5,7] Endosulfan is extremely toxic to fish and aquatic invertebrates and produces strong effects on the nervous system of many organisms, including man.[4,6,8] This neurotoxic action is greater for the α-isomer compared to the β-isomer.[9] Chronic exposure to endosulfan may result in convulsions and behavioural aberration.[9]

Endosulfan is considered as moderately persistent in the soil environment, its two isomers having different degradation times, with half life values of 35 and 150 days in soils under neutral conditions for the α- and β-isomers, respectively.[5] Its dissipation depends on several factors such as volatilization, hydrolysis, microbial degradation and photodecomposition, including the presence of fertilizer, humus content, crop pattern, atmospheric temperature, rain or pollutant concentration.[6,10,11] Water degradation of this compound is rapid, but it can persist for longer times when it is bound to soil particles. Hydrolysis is the main degradation process for endosulfan, the β-isomer being hydrolysed faster than the α-isomer.[4] The principal degradation product in soils and vegetables is endosulfan sulfate, even though other minority breakdown products are also formed such as diol, aldehyde, ether and lactone endosulfan derivatives.[4,6,12]

According to the US Environmental Protection Agency (EPA), endosulfan pesticide levels in food are usually below the tolerance levels; 2 μg g^{-1} being the maximum allowable residue level for lettuce plants.[7] Endosulfan has been detected in several food samples such as vegetables (0.5–13 ng g^{-1}), fruit juices (1–5 ng g^{-1}), tobacco, seafood (0.2–1.7 fg g^{-1}) and milk.[5,13]

Relatively few studies have investigated the plant uptake of organic compounds from contaminated soils. For example, Gonzalez and co-workers have investigated the uptake of organochlorine pesticides (OCPs) by leek,[14] tomato,[15] and lettuce and chard[16] grown on organic farms in the Los Padres Lake watershed in Argentina. In each case OCPs were accumulated in each type of plant studied. The chemical uptake and the distribution into the plant are affected by several factors

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such as physico-chemical properties of the compound, environmental conditions, soil type and plant characteristics (type of root system, shape and chemical characteristics of the leaves, and lipid content).[17] These processes are complex and can be described as a series of consecutive partition reactions, between soil solids and soil water, soil water and plant roots, plant roots and transpiration stream, and transpiration stream and plant stem. Compounds with high K_{OW} values are most likely to be sorbed by the plant root, while chemicals with lower K_{OW} values are likely to be translocated within the plant and may reach the above ground portions of the plant.[17]

Determination of endosulfan residues has usually been carried out by Soxhlet extraction in soils and homogenisation with organic solvents in vegetables, followed by gas chromatography separation with ECD or MS detection.[12] Recently, traditional analytical methods such as Soxhlet or liquid–liquid extraction (LLE) have been replaced by pressurised fluid extraction (PFE),[18] microwave-assisted extraction (MAE)[19] or supercritical fluid extraction (SFE),[20,21] which require less energy, less solvent and provide shorter extraction times.[22,23]

Nowadays, a new trend to evaluate the toxicity of persistent organic pollutants in terms of their potential bioavailability has been identified.[24] Various approaches are available based on the ability to simulate the environmental or human absorption conditions that are likely to lead to the availability of the POP from its matrix. While extensive studies have focused on the availability of POP's from soil[25] little has been done directly related to the potential uptake, and hence bioavailability, of POP's from vegetables.

The aim of this paper is to (a) assess the recovery of endosulfan compounds (α-endosulfan, β-endosulfan and endosulfan sulfate) from soil, (b) evaluate the uptake of endosulfan compounds into lettuce plants grown on contaminated soil, and (c) evaluate the potential for simulated *in vitro* gastrointestinal absorption of endosulfan compounds from lettuce.

2. Experimental

2.1. Apparatus and reagents

A Hewlett Packard gas chromatography HP G1800A GCD (Palo Alto, CA, USA) with a Hewlett Packard HP-5ms capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness), equipped with a quadrupole mass spectrometer detector was used for POP determinations.

A Dionex Accelerated Solvent Extraction ASE™ 200 (Sunnyvale, CA, USA) was used to extract soil and lettuce samples. Hydromatrix supplied by Varian Ltd. (Surrey, UK) was employed for sample drying and sample dispersion during the extraction process.

Standard solutions were prepared in dichloromethane, with α-endosulfan (99.6% w/w), β-endosulfan (99.9% w/w) and endosulfan sulfate (97.7% w/w) provided by Riedel-de Haën (Steinhem, Germany). A TCL Pesticides Mix provided by Supelco (Bellefonte, PA, USA) was used for reference material determination. Pentachloronitrobenzene (PCNB) (99% w/w) was employed as the internal standard (Aldrich, Steinheim, Germany). Acetone and dichloromethane were provided by Fisher Chemicals (Loughborough, UK) and anhydrous Na_2SO_4 by BDH Laboratory Supplies (Poole, UK).

Compost soil (Levington multipurpose compost) and lettuce seedling plants were obtained directly from local markets. A Resource Technology Corporation (Laramie, USA) certified reference material CRM805-050 was employed to assure the quantitative extraction of α-endosulfan and β-endosulfan in soils by the PFE method employed.

For the *in vitro* gastrointestinal extraction a shaking waterbath (Grant Instruments Ltd., OLS 200, Cambridge, UK) was employed. The pH values were adjusted with dilute HCl and NaOH and measured using a pH meter (Jenway 3010, Dunmow, UK). Pepsin-A powder 1 anson unit per g (lactose as diluent) and amylase were provided by BDH Chemicals Ltd. (Poole, UK), bile salts from Sigma Chemicals and pancreatin from Fisher Scientific.

2.2. General procedure: extractions

2.2.1. Soxhlet extraction of soil. In a cellulose extraction thimble, approximately 5 g (accurately weighed) of soil and 5 g of anhydrous Na_2SO_4 were added. The sample was extracted with 220 mL of acetone : dichloromethane 1 : 1 (v/v) for 24 hours. The extract was evaporated under a nitrogen flow to <10 mL, then 20 μL of internal standard of 5 mg mL^{-1} was added. The final extract solution (10.0 mL) was analysed by GC-MS.

2.2.2. Pressurised fluid extraction of soil. Into a 33 mL cell, approximately 8 g (accurately weighed) of soil and hydromatrix were added. The full cell was closed and extracted at 100 °C and 2000 psi, for 10 minutes, with acetone : dichloromethane 1 : 1 (v/v) as solvent. This extract was evaporated under a nitrogen flow to <10 mL, then 20 μL of internal standard was added. The final extract solution (10.0 mL) was analysed by GC-MS.

2.2.3. Pressurised fluid extraction of lettuce. Into a 11 mL cell, approximately 5 g (accurately weighed) of lettuce and hydromatrix were placed. Two sequential extractions were performed at 100 °C and 2000 psi, during 10 minutes, with acetone : dichloromethane 1 : 1 (v/v) as solvent. In order to remove the extracted water 5 g of anhydrous Na_2SO_4 was added post-extraction, this extract was evaporated under a stream of nitrogen to dryness, then 0.5 mL of internal standard (10 μg mL^{-1}) was added and this solution was analysed by GC-MS.

2.2.4. Liquid–liquid extraction of water, gastric and intestinal juice. Aqueous samples were extracted using 3 × 10 mL DCM. The samples consisted of (15 mL) distilled water, gastric juice (0.1% w/v pepsin in saline) and intestinal juice (3% w/v pancreatin, 1% w/v amylase and 0.15% w/v bile salts). Each sample was spiked with endosulfan compounds (all 10 μg mL^{-1}) to assess the influence of sample matrix on recovery. After addition of the internal standard (25 ppm) the extracts were analysed using GC-MS.

2.2.5. *In vitro* gastrointestinal extraction of lettuce. This determination consists of two sequential processes, a gastric and an intestinal digestion, each one carried out employing simulated human conditions (enzymes, pH and temperature). In the first stage, approximately 5 g (accurately weighed) of lettuce (chopped in several pieces) was treated with 15 mL of pepsin (0.1% w/v in saline). The pH of the solution was adjusted to pH 1.8 with diluted HCl. The mixture was then shaken at 100 rpm in a thermostatic bath maintained at 37 °C. The pH was measured every 30 min and maintained less than 2.5 with HCl. After 3 hours, the solution was centrifuged at 3000 rpm for 5 min and filtered.

The second stage involved extraction with intestinal juices. To the gastric digest residue, 5 mL of pancreatin (3% w/v), 5 mL of amylase (1% w/v) and 5 mL of bile salts (0.15% w/v), all in saline solution, were added. Diluted NaOH was used to maintain the pH at ~7 whilst shaking at 100 rpm for 3 hours in the thermostatic bath employing the same conditions as before. All extracts (gastric and intestinal) were extracted with 3 × 15 mL DCM. This was then evaporated under a nitrogen flow to dryness and 0.5 mL of internal standard of 10 μg mL^{-1} was added prior to GC-MS analysis. The resultant sample residue

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was analysed by PFE employing the same conditions as for the lettuce samples.

2.3 General procedure: preparation of soils and lettuce

2.3.1. Preparation of endosulfan contaminated soils for spiking experiments. To determine the influence of soil on the recovery of endosulfan compounds, spiking experiments were performed at (a) different concentrations (10, 20 and 40 μg g^{-1}) and (b) using different spiking procedures (spot, slurry) to investigate the effect of ageing. The effect of concentration was investigated using 25, 50 and 100 μL of a 2000 ppm stock solution of each endosulfan compound which was added to approximately 5 g of soil (accurately weighed). The effect of spiking procedure was investigated using 50 μL of each endosulfan compound. In spot spiking mode, the spiking solution was added directly to the soil in the extraction cell while in slurry spiking mode the spiking solution was added to 40 mL of acetone and then poured over the soil. The solvent was then allowed to evaporate overnight. The influence of endosulfan contact time on the soil was investigated by allowing the slurry spiked soil to age for 1, 6 and 14 days. Each soil sample was extracted using PFE using the procedure described above.

2.3.2. Preparation of endosulfan contaminated lettuces. Contaminated soils at two levels of concentration were used for this study. For the low level, 2 L of acetone containing 8 mg of each endosulfan compound standard was added to 800 g of soil and mixed thoroughly. The high level was prepared in the same manner except with 40 mg of endosulfan standard. The final endosulfan compound concentration in soil was estimated to be 10 and 50 μg g^{-1}, for low and high contamination levels respectively. After air drying for 48 h, the soils were mixed to ensure homogeneity prior to growing the lettuce seedling plants.

Small lettuce seedlings (approximately 10 cm height and 15–20 g) were transplanted into individual pots with 40 g of endosulfan spiked soil. Several lettuces were also planted in unspiked soil as control samples. The lettuce plants were grown over 10, 20 and 33 days. The plants were grown under artificial light with time intervals of 12 hours daylight and 12 hours dark. The air temperature and humidity were monitored over the growing duration. The air temperature was within the range 15.2 to 24.1 °C while the humidity varied between 43 and 79%. The water retention capacity of the soil was experimentally determined to be a minimum of 20 mL, therefore each plant was watered daily with 20 mL of water. No excess water resulted from this process. α-Endosulfan, β-endosulfan and endosulfan sulfate determinations were carried out on the lettuce plants (roots and leaves).

2.4. GC-MS analysis

For GC-MS determinations, an injection volume of 1 μL was employed in split mode (1 : 4). The injector temperature was 250 °C and helium was used as the carrier gas in constant flow mode of 1 mL min^{-1}. The temperature program of the oven was as follows: 60 °C, held for 1 min, increased at a rate of 15 °C min^{-1} to 180 °C, then a second rate of 3 °C min^{-1} to 250 °C and finally held 1 min. The detector temperature was 280 °C and measurements were carried out in selected ion monitoring (SIM) acquisition mode. Retention times and main ions selected for each compound, with their relative abundances, are summarized in Table 1. Detector tune tests were performed daily with perfluorotributylamine.

3. Results and discussion

3.1. Analytical features of persistent organic pollutant determination

Calibration curves were established with five standards, with concentrations ranging from 2 to 20 μg mL^{-1} using an internal standard of concentration 10 μg mL^{-1}. Limit of detection values were established using the expression $3s_{blank}/b$, where s_{blank} is the standard deviation of five measurements of a standard solution of 2 μg mL^{-1} and b the slope of the calibration curve. Table 1 shows the limit of detection values obtained for each compound. The detector response was linear over the range of concentration studied, with correlation coefficients ranging from 0.979 to 0.999. Limits of detection in soil were 0.6, 0.4 and 0.5 μg g^{-1} for α-endosulfan, β-endosulfan and endosulfan sulfate, respectively and 0.05, 0.03 and 0.04 μg g^{-1} in lettuce determinations.

3.2. Extraction of POP's compounds from soil

The first step was the development of an approach for the determination of POP's in soils, based on PFE. Previously determined optimal conditions for extraction of POP's from soil were employed.[26] A certified reference material was analysed by Soxhlet extraction and by PFE in order to assess the quantitative extraction of these compounds from soil. Pressurised fluid extraction uses a smaller amount of solvent than Soxhlet and provides quicker extractions. In this procedure a pre-concentration step was needed to increase sensitivity. It involved subjecting the extract to a nitrogen flow in order to achieve partial solvent evaporation. Recovery tests were carried out to check that minimal compound losses occurred under the cited conditions. Typical recoveries ranged from 82% for DDT to 94% for methoxychlor after Soxhlet extraction (solvent volume reduced from 220 mL to <10 mL) and 83% for α-endosulfan to 95% for endrin aldehyde after PFE (solvent volume ~ 50 mL reduced to <10 mL). No correction was made to subsequent data to adjust for these solvent evaporation losses. The recovery of POP's from the certified reference material, CRM 805-050, as determined by Soxhlet and PFE methods followed by GC-MS are shown in Table 2. In each case the results, by each extraction technique, were in agreement with certificate values. It should be noted that endosulfan sulfate was not present in the CRM. On that basis PFE was used for subsequent determination of POP's from soil.

3.3. Endosulfan ageing in soil

For this contaminant uptake study in lettuces, endosulfan compounds were the only persistent organic pollutant studied, owing to their wide use in vegetable crops. The endosulfan compounds studied were the α- and β-isomers, and their major metabolite, endosulfan sulfate.

In order to test the quantitative recoveries of endosulfan compounds at different concentration levels in soil, PFE was performed on spiked soil at three concentrations (10, 20 and 40 μg g^{-1}). Results shown in Table 3(A) indicate that quantitative recoveries can be obtained from slurry spiked soil irrespective of concentration. Soil blanks were also extracted and analysed to ensure that no other chlorinated insecticides or majority compounds were present, which may affect the endosulfan degradation and lettuce uptake.[11] Different ways of soil spiking were studied in order to investigate the influence of soil organic matter on the retention of endosulfan compounds. The results, shown in Table 3(B), indicate the influence of soil ageing on the recovery of endosulfan compounds from slurry spiked soil samples. The lowest recoveries were obtained from the slurry spiked soil which had been aged for 14 days indicating the influence of soil organic matter on the retention of endosulfan compounds.

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Table 1 GC-MS parameters and limits of detection for persistent organic pollutants determination

Compound	RT	Quantifier ion (m/z, %)	Qualifier ion (m/z, %)	LOD/μg mL^{-1} in solution	LOD/μg g^{-1} in soil	LOD/μg g^{-1} in lettuce
Lindane	13.84	108.95 (100)	180.90 (98)	0.3	0.4	0.03
α-Endosulfan	21.01	194.90 (100)	169.90 (75)	0.5	0.6	0.05
DDE	22.24	245.95 (100)	246.95 (60)	0.6	0.7	0.06
Endrin	23.29	67.15 (100)	81.05 (34)	0.4	0.5	0.04
β-Endosulfan	23.75	194.90 (100)	158.90 (80)	0.3	0.4	0.03
DDD	24.31	235.05 (100)	237.50 (64)	0.3	0.4	0.03
Endrin aldehyde	24.72	67.05 (100)	249.85 (21)	0.6	0.7	0.06
Endosulfan sulfate	25.85	271.75 (100)	228.85 (80)	0.4	0.5	0.04
DDT	26.22	235.05 (100)	236.95 (64)	0.5	0.7	0.05
Methoxyclor	29.37	227.15 (100)	228.15 (17)	0.4	0.5	0.04
PCNB[a]	13.95	141.95 (100)	236.80 (90)	—	—	—

[a] Internal standard.

3.4. Endosulfan total determination in lettuce

Pressurised fluid extraction was carried out to assess the total recovery of each endosulfan compound from spiked lettuce samples. Both leaf surface and stems were spiked with endosulfan compounds at a concentration in lettuce of 33 μg g^{-1}. The influence of PFE on the recovery of endosulfan compounds was evaluated. This was done by carrying out three successive extractions on the same spiked lettuce sample to assess the completeness of recovery. The results are shown in Table 4. It is observed that two extractions are needed for exhaustive recovery of endosulfan compounds from spiked lettuce samples. It is also observed that quantitative recovery is not obtained (average recoveries range from 72 to 77%). This may be attributable to degradation within the lettuce matrix or losses that occur during the spiking process.

3.5. Uptake of endosulfan compounds by lettuce

The uptake of endosulfan compounds by lettuce grown on contaminated soil (10 and 50 μg g^{-1}) was determined. Control lettuce plants were also grown on unadulterated soil. Lettuce plants were harvested at 10, 20 and 33 days. The results are shown in Table 5. In accordance with expectations it is noted that endosulfan compounds are detected in the roots of lettuce plants even after 10 days. However, a growing period of 33 days on the most contaminated soil (50 μg g^{-1}) is required before any endosulfan compounds are detected in the leaves. The root to leaf ratio was found to be 3.1 for α-endosulfan, 46.0 for β-endosulfan, and 24.3 for endosulfan sulfate. It is also noted that during the growing period 10–33 days the amount of endosulfan compounds in the roots of the lettuce plants increases. Similar results have been reported for leeks[14] and tomatoes[15] grown on organic farms (no direct application of pesticides) in Argentina over short, medium and long term growth periods. In this work[14,15] endosulfan compound residues were monitored in the roots and leaves of leeks and tomatoes. In the case of leek plants,[14] endosulfan compound residues were always the highest in the root for younger plants (15 and 59 days). In the case of leek plants at maturity (210 days) the highest levels were found in the leaves. These findings were in contrast to the data presented for tomatoes[15] where the highest endosulfan compound residue levels were found in the leaves at 15, 59 and 151 days. In the case of residue levels of endosulfan compounds in the leek and tomato studies significantly higher concentrations of endosulfan sulfate were always found. This was reported to be due to the fact that both α-endosulfan and β-endosulfan metabolise within the plant to endosulfan sulfate.[14,15]

Table 2 Determination of POP's in a certified reference soil sample (CRM 805-050) using either Soxhlet extraction or PFE followed by GC-MS

	Persistent organic pollutant concentration/μg g^{-1}		
Compound	Reference value[a]	Soxhlet extraction (mean ± SD, $n = 3$)	PFE (mean ± SD, $n = 3$)
Lindane	11 ± 5	11.5 ± 0.5	10.2 ± 0.8
α-Endosulfan	7 ± 4	3.2 ± 0.6	2.9 ± 0.3
DDE	19 ± 9	26.6 ± 0.6	23.3 ± 0.5
Endrin	13 ± 8	18.3 ± 0.4	20.9 ± 0.8
β-Endosulfan	6 ± 3	4.3 ± 0.9	3.7 ± 0.6
DDD	20 ± 9	22.0 ± 0.5	17 ± 1
Endrin aldehyde	0.1 ± 0.2	<LOD[b]	<LOD[b]
DDT	0.8 ± 0.3	<LOD[b]	<LOD[b]
Methoxyclor	16 ± 8	17.7 ± 0.3	15.1 ± 0.9

[a] Resource technology corporation. [b] LOD = limit of detection.

3.6. Endosulfan stability using *in vitro* gastrointestinal extraction

In vitro gastrointestinal extraction consists of two procedures that simulate human digestion. The first stage involves extrac-

Table 3 Influence of (A) concentration and (B) spiking procedure and ageing on the recovery of endosulfan compounds from soil using PFE-GC-MS

(A)	Influence of concentration on recovery (%) from slurry spiked soil		
	α-Endosulfan	β-Endosulfan	Endosulfan sulfate
10 μg g^{-1}	89.0 ± 0.9	88.1 ± 3.3	95.0 ± 3.5
20 μg g^{-1}	90.0 ± 1.9	88.5 ± 3.2	94.1 ± 4.0
40 μg g^{-1}	95.6 ± 1.3	94.3 ± 1.1	101.6 ± 2.6

(B)	Influence of soil spiking procedure and ageing on the recovery (%) of endosulfan compounds (spiking concentration 20 μg g^{-1})		
	α-Endosulfan	β-Endosulfan	Endosulfan sulfate
Direct spiking	90.0 ± 1.9	88.5 ± 3.2	94.1 ± 4.0
Slurry spiking	85.7 ± 7.4	84.6 ± 6.6	92.1 ± 9.2
Slurry spiking and ageing for 6 days	76.9 ± 2.8	75.0 ± 3.7	82.7 ± 5.9
Slurry spiking and ageing for 14 days	74.9 ± 4.1	72.8 ± 1.4	77.1 ± 2.5

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Table 4 Influence of PFE on the recovery of endosulfan compounds from spiked lettuce samples[a]

	Cumulative recovery (mean % ± SD, $n = 3$)					
	From leaves			From stem		
Compounds	1st extract	2nd extract	3rd extract	1st extract	2nd extract	3rd extract
α-Endosulfan	69.8 ± 11.5	71.9 ± 8.4	71.9 ± 8.4	59.7 ± 8.0	71.5 ± 6.6	71.5 ± 6.6
β-Endosulfan	70.5 ± 10.3	74.2 ± 6.3	74.2 ± 6.3	60.8 ± 10.3	72.1 ± 6.9	72.1 ± 6.9
Endosulfan sulfate	63.3 ± 11.5	76.8 ± 9.4	76.8 ± 9.4	64.4 ± 9.3	76.4 ± 6.4	76.4 ± 6.4

[a] Spike level 33 μg g^{-1}.

Table 5 Uptake of endosulfan compounds by lettuce plants grown on contaminated soil followed by PFE-GC-MS

		Endosulfan concentration (mean ± SD, $n = 3$)/μg g^{-1}					
		Day 10		Day 20		Day 33	
Endosulfan concentration in soil/μg g^{-1}	Compound	Root	Leaf	Root	Leaf	Root	Leaf
10 μg g^{-1}	α-Endosulfan	0.3 ± 0.2	<LOD[a]	1.4 ± 0.7	<LOD[a]	1.7 ± 0.3	<LOD[a]
	β-Endosulfan	0.12 ± 0.08	<LOD[a]	1.0 ± 0.6	<LOD[a]	1.5 ± 0.3	<LOD[a]
	Endosulfan sulfate	0.14 ± 0.06	<LOD[a]	1.2 ± 0.5	<LOD[a]	1.5 ± 0.4	<LOD[a]
50 μg g^{-1}	α-Endosulfan	0.5 ± 0.3	<LOD[a]	1.7 ± 0.8	<LOD[a]	2.5 ± 0.6	0.8 ± 0.004
	β-Endosulfan	0.4 ± 0.2	<LOD[a]	0.8 ± 0.3	<LOD[a]	2.3 ± 0.6	0.05 ± 0.01
	Endosulfan sulfate	0.5 ± 0.2	<LOD[a]	1.2 ± 0.4	<LOD[a]	1.7 ± 0.4	0.07 ± 0.01

[a] <LOD = less than the limit of detection.

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tion with gastric juice (pepsin) to simulate the activity of the stomach, while stage two involves extraction with intestinal juice (pancreatin, amylase and bile salts) that simulates the activity of the intestines. These extractions were performed in a thermostatic bath at 37 °C with agitation during several hours. After this, liquid–liquid extraction of the gastric and intestinal extracts were carried out to determine the recovery of endosulfan compounds. Recovery tests of these processes were performed in order to assess the potential for decomposition and losses of some endosulfan compounds due to the presence of enzyme(s) and the influence of pH. Table 6 shows the recoveries of endosulfan compounds by liquid–liquid extraction in gastric juices and intestinal juices. Extraction of endosulfan compounds from distilled water was done as a control to enable the effect of the *in vitro* gastrointestinal extraction to be investigated. It is noted (Table 6) that recoveries in gastric and intestinal juices range from 82.1–86.1% which is comparable to the recoveries of endosulfan compounds from distilled water (88.5–89.9%).

3.7. Determination of endosulfan bioavailability in spiked lettuces

Assessment of the potential of gastrointestinal extraction to determine the bioavailability of endosulfan compounds required the use of spiked lettuce leaves. Lettuce leaves were spiked at a concentration of 10 μg g^{-1}. The lettuce leaves were then subjected to *in vitro* gastrointestinal extraction. The resultant lettuce residue was then extracted using PFE. The results are shown in Table 7. The results indicate that the bioavailability of endosulfan compounds in spiked lettuce leaves is minimal (<3.5% bioavailability). The majority of the endosulfan compounds were not extracted by the *in vitro* gastrointestinal extraction approach, but remained within the lettuce matrix and were recovered by PFE. The total recovery by *in vitro* gastrointestinal extraction and PFE of the residual fraction amounted to approximately 35%. This non-quantitative recovery was attributable to losses caused by the transfer

Table 6 Liquid–liquid extraction of endosulfan compounds[a] from aqueous solution, gastric juice and intestinal juice followed by PFE-GC-MS

	Recovery (mean % ± SD, $n = 5$) based on 3 sequential extractions		
	Water	Gastric juice at pH 2.5	Intestinal juice at pH 7
α-Endosulfan	89.9 ± 0.12	82.1 ± 0.21	85.0 ± 0.20
β-Endosulfan	88.5 ± 0.19	86.1 ± 0.19	83.5 ± 0.22
Endosulfan sulfate	88.6 ± 0.34	85.4 ± 0.33	85.1 ± 0.20

[a] Spike level 10 μg mL^{-1}.

Table 7 Recovery of endosulfan compounds from spiked lettuce leaves[a] using PFE-GC-MS and an assessment of the bioavailability of endosulfan compounds using simulated *in vitro* gastrointestinal extraction followed by LLE-GC-MS

	Recovery (mean ± SD, $n = 3$) following *in vitro* gastric extraction/μg g^{-1} LLE-GC-MS	Recovery (mean ± SD, $n = 3$) following *in vitro* intestinal extraction/μg g^{-1} LLE-GC-MS	Residual fraction[b] (mean ± SD, $n = 3$)/μg g^{-1} PFE-GC-MS	Total recovery after G + I + residual fraction/μg g^{-1}
α-Endosulfan	nd	0.06 ± 0.02	3.51 ± 0.64	3.56
β-Endosulfan	nd	0.12 ± 0.02	3.69 ± 0.76	3.81
Endosulfan sulfate	nd	0.09 ± 0.01	3.35 ± 0.72	3.44

nd = not detected.[a] Spiking level was 10 μg g^{-1}. [b] Reported on a dry weight basis (μg g^{-1}).

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of lettuce material from the liquid–liquid extraction stage to the PFE stage.

The simulated *in vitro* gastric intestinal extraction was based on 3 hours gastric and 3 hours intestinal extraction. In the case of gastric extraction simulating activity in the stomach this represents the upper limit for food to be present. Typically, food is retained in the stomach from between 8 min to 3 hours. In the case of the intestinal extraction food samples can be present in the duodenum, jejunum and ileum for between 30 min, 1.5 hours and 4–5 hours, respectively. Therefore in this procedure, lettuce samples are subjected to approximately 50% of the potential time for intestinal absorption. In principle therefore, levels of intestinal extraction could double (increase by 50%) which would equate to $<7\%$ bioavailability (0.034 μg g^{-1} for α-endosulfan, 0.065 μg g^{-1} for β-endosulfan, and 0.054 μg g^{-1} for endosulfan sulfate). These upper estimates of bioavailability from lettuce plants are well within the EPA tolerance level of 2 μg g^{-1} for the maximum allowable residue level for lettuce plants.[7]

4. Conclusion

The uptake of α-endosulfan, β-endosulfan and endosulfan sulfate by lettuce plants from contaminated soil has been found to be minimal (up to 2.5 μg g^{-1} in roots and up to 0.8 μg g^{-1} in leaves) even when grown on contaminated soil (10 and 50 μg g^{-1}). An assessment of the bioavailability of the endosulfan compounds using *in vitro* gastrointestinal extraction was carried out. It was found that a minimal amount of endosulfan compounds were determined to be available for absorption in the gut, based on the simulated extraction procedure.

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